

Streptococcus suis : genome based approaches to identify virulence factors and vaccine candidates  
 Hilde Smith, Alex Bossers, Herma Buys & Astrid de Greeff  
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*Streptococcus suis* : genome based approaches to identify virulence factors and vaccine candidates


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 Funding: EADGENE, RD bioinformatics CVI



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*Streptococcus suis*

- meningitis
- arthritis
- sepsis



- adult pigs carrier
- high percentage (>80%) herds infected  
10% infected herds acute *S. suis* problems
- infected herds: antibiotic treatment/autovaccines

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*Streptococcus suis*


- Zoonotic disease: infections in humans (meningitis)
- Western countries: human infections occasionally observed
- Asia: important human pathogen
  - Outbreak in China
  - Patients with signs of toxic shock like
  - Emergence more virulent isolate
- Control *S. suis* infections in pigs as well as in humans important



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Control of disease hindered

- S. suis* population very heterogeneous
- Several serotypes cause disease
  - Europe: 1, 2, 7 and 9
  - USA: 2, 3, 7, 8
  - South-East Asia: human 2
- Several serotypes and genotypes carried by one animal simultaneously
- Differences in virulence between as well as within serotypes
- No effective vaccines available



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Study relationship between gene content, serotype and virulence: genome wide approaches

- Comparative Genome Hybridization (CGH)
- Whole genome analysis of isolates belonging to various serotypes: differ in virulence
- Complementation approach

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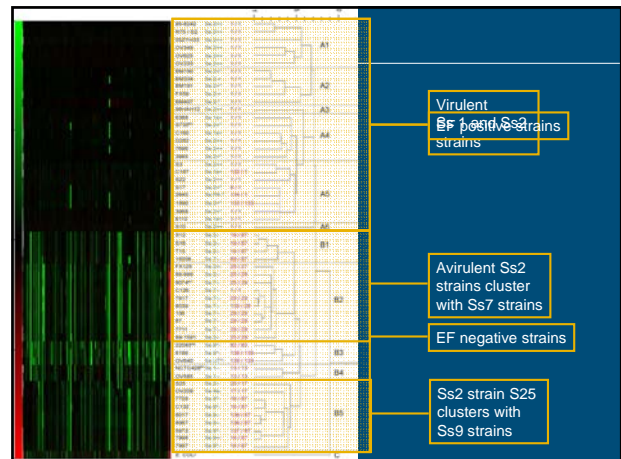
Comparative genome hybridisation (CGH)

- Oligoarray designed based on whole genome sequence virulent serotype 2 isolate (pig) → CGH
- Each ORF is represented by 2-4 probes
- Study diversity in gene content by DNA-DNA hybridisation
- Yields information of a one-way character: genes that are unique to the tester strain cannot be detected (since they are not present on array)

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### Streptococcus suis strains (n=55)

- Serotype 1: 6 strains
- Serotype 2: 29 strains
  - Differ in virulence: avirulent, virulent, weakly virulent
  - Pig isolates as well as human isolates (Vietnam and China)
- Serotype 7: 8 strains
- Serotype 9: 9 strains
- Three other isolates (different serotypes)
- Control: *E. coli*


### CHG data showed

- Extensive sequence variation between serotypes
- EF is a marker for virulent serotype 1 and 2 isolates
- Clustering of virulent serotype 1 and 2 isolates
  - Subclustering of human isolates from Vietnam
- Avirulent Ss2 strains have Ss7 backbone:
  - Capsular exchange between Ss2 and Ss7 strains
- Avirulent Ss2 has Ss9 backbone
  - Capsular exchange between Ss2 and Ss9 strains



### CGH

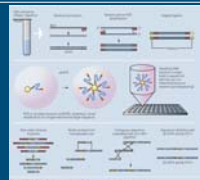

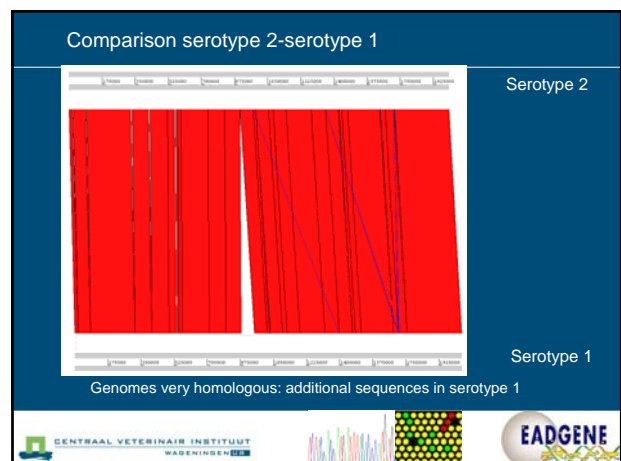
- Disadvantage CGH → Only 1 genome on array
- no information on additional genes → extension of array with new genomes / serotypes interesting!
- Whole genome sequences available from serotype 2 isolates only
- Determined: whole genome sequences of serotype 1, 7 and 9 isolates



### Whole genome sequences serotype 1, 7 and 9



454 sequencing technology

- Assembled data (de novo):
  - serotype 1 isolate: 28 contigs
  - serotype 7 isolate: 138 contigs
  - serotype 9 isolate: 135 contigs
- Created artificial genomes by joining the contigs
- Contigs were joined by using a linker containing start/stop codons in all 6 reading frames
- Genome alignments of artificially joined chromosome were studied using Artemis Comparison Tool (Sanger tool)

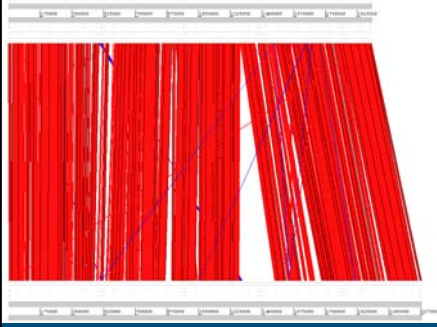




### Serotype 1 genome


- Genomes serotype 1 and 2 very similar; additional sequences in serotype 1 genome
- Experimental infections showed that virulence of serotype 1 isolates >> virulence serotype 2
- Future research
  - Capsule??
  - Genes in additional sequences??
  - SNPs??

### comparison serotype 2- serotype 9 genomes

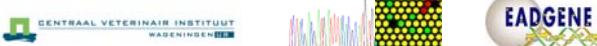


Considerable differences




### Serotypes 7 and 9

- Considerable differences between serotype 2 genome and serotypes 9 genomes
  - Insertions as well as deletions
  - Serotype 9: at least 12 insertions: 4-179 kb
- Experimental infections showed that
  - virulence of serotype 2 > virulence serotype 9
  - Colonization tonsil serotype 9 > serotype 2
- Future research
  - Identify regions important for differences in virulence
  - All additional genes will be used to update the array:
    - CGH studies
    - Transcriptome studies



### Complementation approach



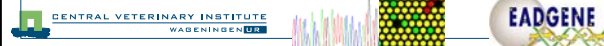
Serotype 2: pathogenic and weakly pathogenic isolate closely related

Plasmid library: genomic DNA pathogenic strain (> 3kb)


Introduced into weakly pathogenic strain

Pigs infected with library (i.v.)



Select for genetic determinants of pathogenic strain which can increase virulence of weakly pathogenic strain



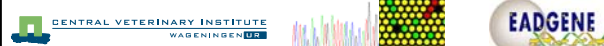
### Complementation approach



Strain	No. of pigs	Dose of <i>S. suis</i> (CFU)	Mortality <sup>a</sup> (%)	Mean no. of days until death	Morbidity <sup>b</sup> (%)
S735(pCOM-L)	4	10 <sup>7</sup>	100	4	100
	4	10 <sup>8</sup>	75	7	100

One specific clone enriched in 7 pigs  
 Enrichment is not tissue specific

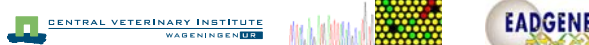


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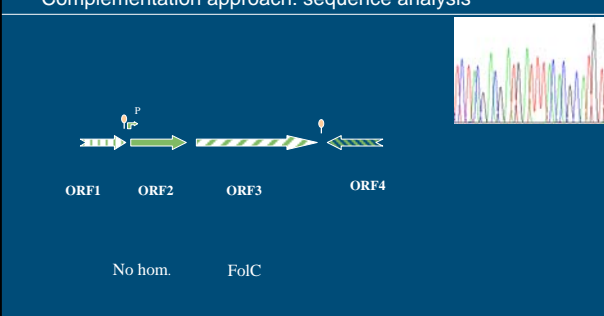
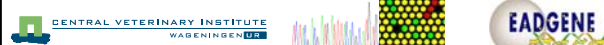
### Complementation approach

Strain	No. of Pigs	Dose of <i>S. suis</i> (CFU)	Mortality* (%)	Mean no. of days until death
S735(pCOM-L)	4	10 <sup>7</sup>	100	4
	4	10 <sup>8</sup>	75	7
S735(pCOM-V10)	5	10 <sup>6</sup>	100	1
S735(pCOM1)	4	10 <sup>6</sup>	25	12

- Introduction of a fragment V10 from a pathogenic strain into the weakly pathogenic strain transformed the weakly pathogenic strain into a highly pathogenic strain
- Resulting strain more pathogenic than the original strain
- Protein(s) encoded by the fragment are important virulence factor(s)

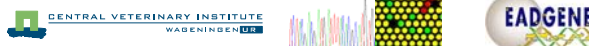


### Complementation approach: sequence analysis

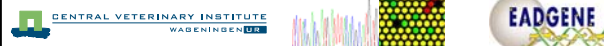
### Virulence enhancing effect caused by ORF2 or by ORF3 protein?

Strains	no of pigs	dose	mortality	mean no. of days till death	no of pigs in which <i>S. suis</i> was isolated from CNS	no of pigs in which <i>S. suis</i> was isolated from joints
S735 (pCOM-V10)	4	10 <sup>6</sup>	100	1	4	4
S735 (pCOM-ORF2)	4	10 <sup>6</sup>	100	1	4	4
S735 (pCOM-ORF3)	4	10 <sup>6</sup>	0	11	0	0
S735 (pCOM1)	4	10 <sup>6</sup>	0	11	0	0




### Complementation approach

- ORF2 is an important virulence factor for *S. suis*
- Small membrane protein; expression in *E. coli* is lethal
- Unable to obtain *S. suis* mutants in ORF2
- Transcriptome analysis will be used to study the role in virulence in more detail



### Summary

- Different approaches resulted in the identification of different virulence factors
- Future: role of these proteins will be tested in experimental infections/vaccine candidates



### Questions

